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Bioremediation on the Shore after an Oil Spill from the *Nakhodka* in the Sea of Japan. III. Field Tests of a Bioremediation Agent with Microbiological Cultures for the Treatment of an Oil Spill

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We conducted a field test to confirm the effectiveness of a seed culture of petroleum-degrading bacteria, TerraZyme™ (Oppenheimer Biotechnology) for enhancing the biodegradation of heavy oil spilled from the *Nakhodka* on the shore. We defined two designated sites: one for treatment, and a second site 40 m distant as an untreated control site for the experiments. In each site, we prepared the rocks and concrete blocks polluted by the oil, by placing them in wire cages on the beach. The experiments were carried out for eight weeks. Once a week, we photographed the rocks and concrete blocks, treated their surfaces with TerraZyme™, and determined the coverage of remaining oil on the substrates from the photographs using digital photographic image analysis. The test results clearly show that TerraZyme™ is able to significantly enhance the biodegradation of oil on the shore.

Keywords: ***Nakhodka*; oil spill; bioremediation; microbiological cultures; TerraZyme™.**

Introduction

In January 1997, a Russian tanker, *Nakhodka*, broke up on the coast of the Sea of Japan releasing an estimated 19 000 t of heavy crude oil. The oil came onto the sandy beach, rocky shore, port, and other areas, as platter-sized globules. The resultant oil pollution ranged over 12 000 km along the rocky coast, shoreline structures and sandy beaches. The oil was removed from the shore by the hands of innumerable volunteers, treated with chemical agents (oil dispersants).

In a small town in Kasumi-cho, Kinosaki-gun, Hyogo prefecture, a bioremediation agent with microbiological cultures, TerraZyme™ (Oppenheimer Biotechnology) was applied by the members of a local fishery cooperative association. They scattered approximately 250 kg of the bioremediation agent on the oil attached to shoreline rocks and concrete in the small fishing harbour of the town. The visual and video observations after treatment with the bioremediation agent indicated an accelerated loss of oil attached to shoreline rocks and concrete, as compared to untreated areas (Tsuneaki Terakawa, pers. comm.). However, this observation was qualitative. In fact, all previous studies of bioremediation agents with microbiological cultures for the treatment of oil spills in the field have been qualitative, not quantitative (Venosa *et al.*, 1991; New Scientist, 1990; Mauro and Wynne III, 1990; Nadeau *et al.*, 1991). A more quantitative experiment was required to confirm the effectiveness of the bioremediation agent (Atlas, 1995; Higashihara, 1998).

To quantitatively evaluate the effectiveness of the seed cultures of petroleum degrading bacteria, we developed a technique to measure the coverage of oil on the substrate with a digital photographic image analysis

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running on a personal computer. Using this technique, we conducted field tests to confirm the effectiveness of TerraZyme™ on the enhancement of biodegradation of the heavy oil from the *Nakhodka* that adhered to the rocks and concrete blocks on the shore around the Kasumi-cho area.

In this paper, we will report the results of the field experiments and discuss the use of seed cultures of petroleum-degrading bacteria for the treatment of oil pollution following oil spills.

Materials and Methods

The study area was located on the Kasumi-cho shoreline (134°43′E, 35°42′) (Fig. 1), which was highly impacted with heavy oil spilled from the *Nakhodka* in January 1997, in the Sea of Japan at Minagai. We defined a treatment site and an untreated control site, 40 m distant, for the bioremediation experiments on the *Nakhodka* heavy oil, where water exchange between the two sites was restricted by massive rocks between these two sites. At each site, we prepared three sets of rocks which each had at least one 200 cm^2 flat face coated by the heavy oil from the *Nakhodka*, and duplicates of concrete blocks (20 × 20 cm), which were painted with the heavy oil from the *Nakhodka*. These rocks and concrete blocks were then placed within the wire cages (62 × 42 × 32 cm) and anchored at the intertidal zone on the beach, where the rocks and blocks were frequently washed by the waves (Fig. 2). The experiments were carried out for eight weeks, from 9 July 1997 to 4 September 1997. Once a week, the wire cages with the rocks and concrete blocks were carried to a higher point on the beach. The rocks and concrete blocks were removed from the wire cages, and each was photographed at a distance approximately 50 cm. Prior to taking pictures, if the surface of the rocks and concrete blocks was wet, we removed the water with a blotter or left them until they dried. Water drops on the rocks tended to reflect light and appeared in the photographs as white dots, even if these parts were actually coated by black-coloured oil. After taking pictures of the rocks and concrete blocks, they were treated with a bioremediation agent with microbiological cultures, TerraZyme™ (Oppenheimer Biotechnology) by sprinkling the powder of TerraZyme™ over them at a density of 100 g/m^2. After treatment, the rocks and concrete blocks were returned to the wire cages, and the cages set at the water's edge on the beach again.

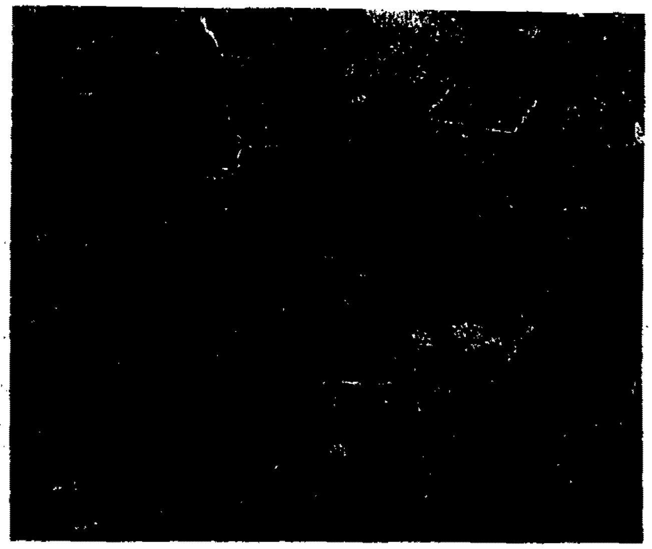

Fig. 2 The wire cages with the rocks and concrete blocks anchored at the intertidal zone on the beach.

The photographs of the rocks and concrete blocks were imported to a personal computer (Power Macintosh 7300, Apple) via flatbed scanner (GT6500,

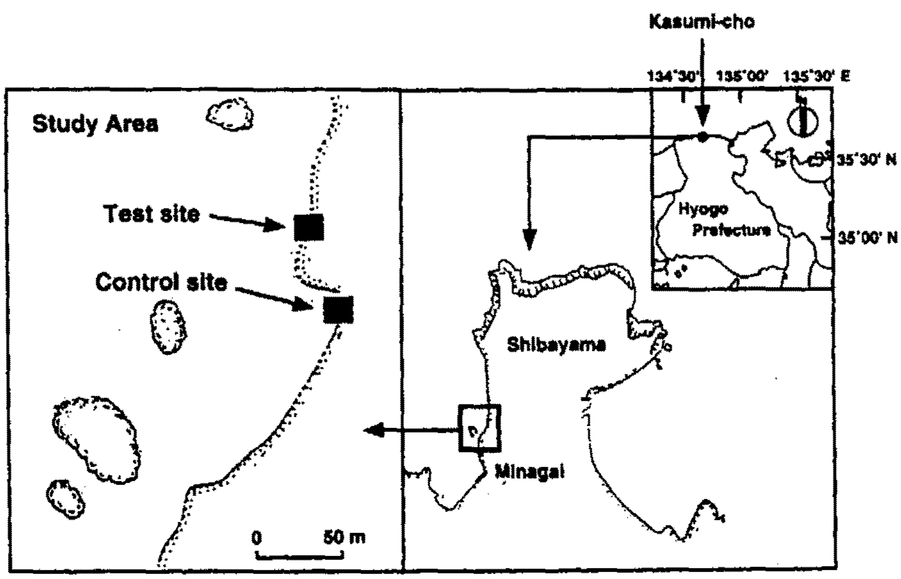

Fig. 1 The study area; Minagai, Kasumi-cho, Kinosaki-gun, Hyogo prefecture, Japan.

EPSON), and then converted to monochrome digital photographic images with 256 gradations of gray. The pixels in the digital images were classified into three different groups according to the darkness of their grey colours (black pixels in Group 1, grey ones in Group 2 and white ones in Group 3, respectively), using a technique of digital photographic image analysis with the personal computer system and NIH Image Ver. 1.60 (software for image analysis) (Fig. 3). The black pixels in Group 1 represented the areas which were thickly coated by the oil. The white pixels in Group 3 indicated the areas with the original rock and concrete block colours, and those areas which were absent of an oil coating. The grey pixels in Group 2 showed the areas where the oil coating was partly lost from the substrates. With the digital image analysis system, we counted the number of these three groups of pixels in the digital images of the rocks and concrete blocks respectively, and calculated the percentage of coverage of these three groups of pixels on the images of rocks and concrete blocks in order to evaluate the amount of oil remaining on them.

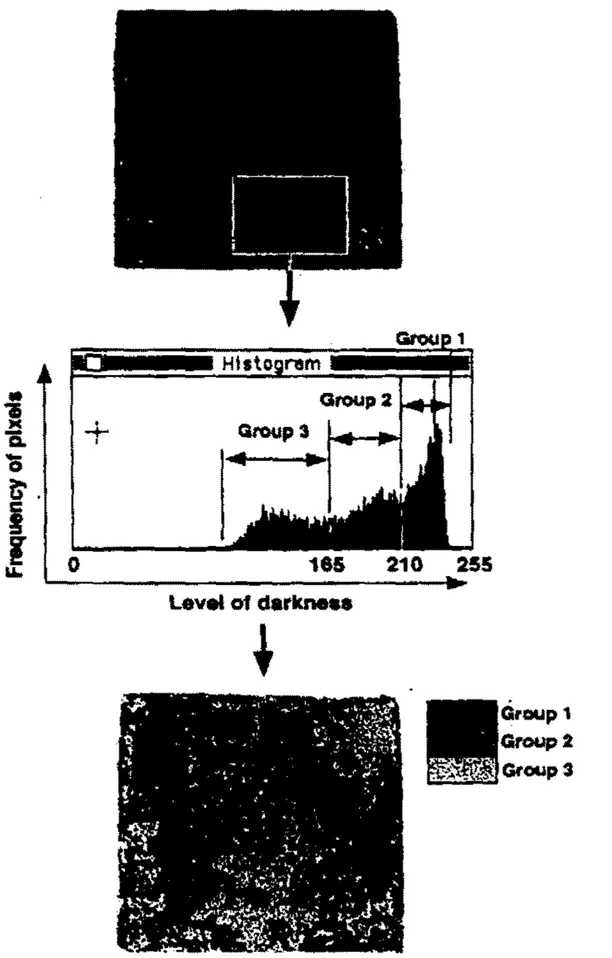

Fig. 3 Processing of a picture, using a technique of digital photographic image analysis with the personal computer system. All of the pixels in the digital image of the picture were classified into three different groups according to the darkness of their grey colours.

Results

Fig. 4 shows the photographic digital images of the rocks and concrete blocks at (a) the treated and (b) untreated sites at the beginning and end of the oil treatment test with TerraZyme™. These images were processed with the digital image analysis system, and expressed with three different colours of pixels (the pixels of Group 1–3) to clearly show the distribution of oil on them.

At the start of the oil treatment test, the images of the rocks and concrete blocks in both of the treated and untreated sites were made up of mostly black pixels (Group 1). At the end of the test, there were distinct differences in the distribution of three groups of pixels in the images of the rocks and concrete blocks between the two sites. At the treated site, the majority of the images were replaced by grey pixels of Group 2 or white ones of Group 3. At the untreated site, the number of white pixels of Group 3 increased as well, in all of the images, but the majority of pixels in these images remained black (Group 1) pixels.

Fig. 5(a) compares the percentage of coverage of black pixels (Group 1) on the images of rocks between the treated and untreated sites during the period of the oil treatment test. At the start of the oil treatment test, the percent coverage on the rocks was $91.0 \pm 6.6\%$ (mean ± S.D.) at the treated site and $87.0 \pm 8.2\%$ (mean ± S.D.) at the treated site, respectively. The difference between them was not statistically significant (ANOVA, $p > 0.5$). The percent coverage at the treated site decreased faster than those at the untreated site during the period of the test. Eight weeks from the start of the test, the percentage of coverage at the treated site decreased to $13.7 \pm 8.2\%$ (mean ± S.D.), which was significantly lower than that at the untreated site ($51.0 \pm 16.1\%$, mean ± S.D.) (ANOVA, $p < 0.05$).

The effectiveness of TerraZyme™ was even more evident on the treatment of oil that coated the concrete blocks. As shown in Fig. 5(b), at the treated site, the percentage of coverage rapidly decreased in the first three weeks and fluctuated within a range of approximately between 2% and 11%, 6–8 weeks later. At the untreated site, the percentage of coverage never dropped below 50% throughout the period of the treatment test.

Discussion

The results of the present study clearly show that TerraZyme™ is able to significantly enhance the biodegradation of oil in the field. Due to the effectiveness of TerraZyme™, at the end of the eight-week treatment test, the initial black-coloured oil nearly disappeared from the surface of the treated rocks and concrete blocks, while more than 50% of the surface areas was still left black on the untreated ones. It was very difficult to determine the precise ingredients of the oil which adhered to the rocks and concrete blocks on the shore. However,

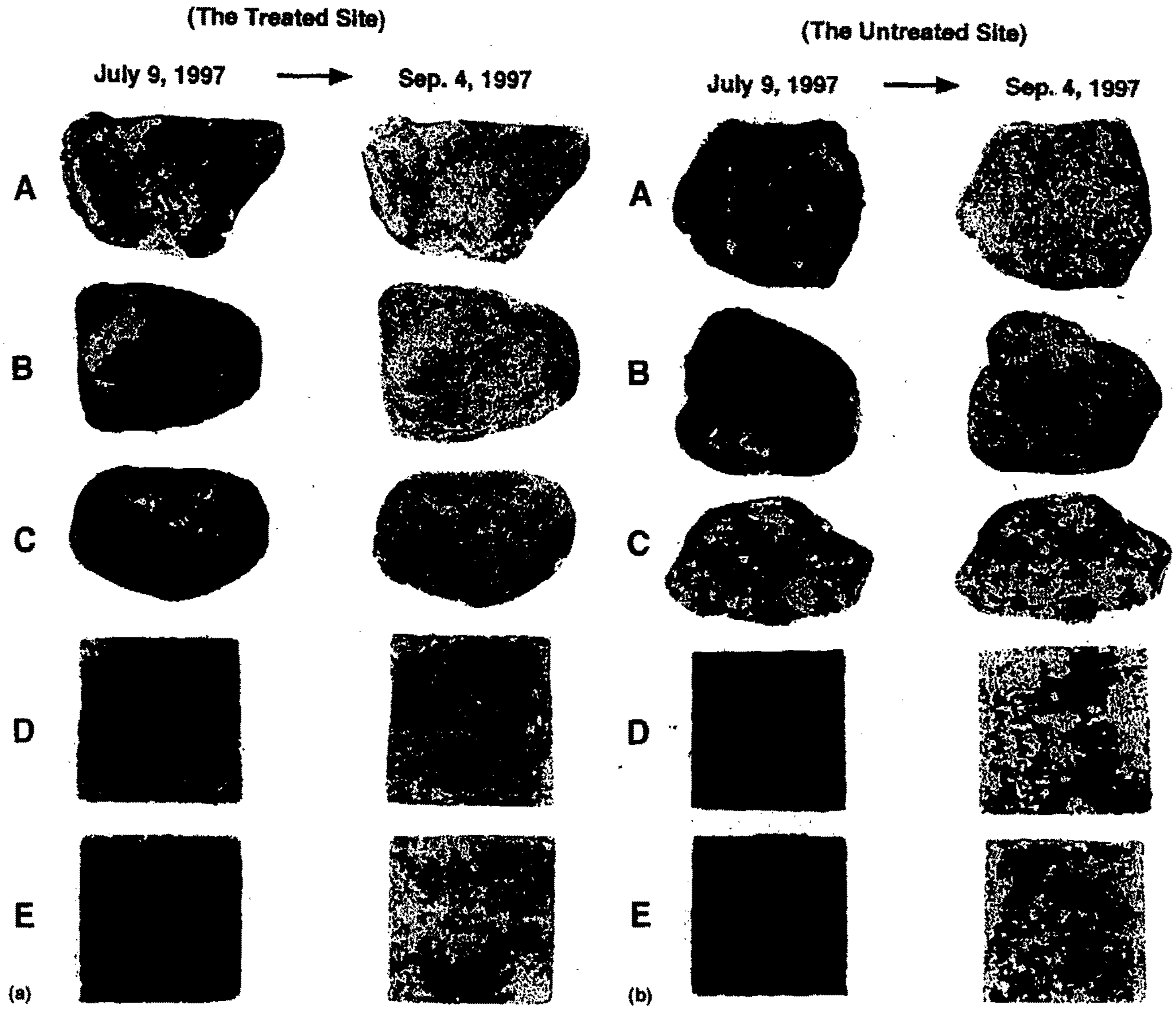

Fig. 4 The photographic digital images of the rocks and concrete blocks expressed with three different groups of pixels according to the darkness of their grey colours at (a) the treated and (b) untreated sites at the begining and end of the oil treatment test with TerraZyme™.

the appearance of the untreated rocks and concrete blocks coated by black-coloured oil indicated that the hardest portions of oil for biodegradation such as asphaltum, resin etc., remained adhered to the surface. The natural petroleum-degrading bacteria that occur at the sites of oil pollution tend to possess restricted potentials for biodegradation of oil to only the most easily decomposable portions, such as saturated hydrocarbons and small molecules of aromatics (Leahy and Colwell, 1990; Atlas and Bartha, 1992; Higashihara, 1998). Therefore, as long as the degradation of heavy oil relies only on the natural degradation processes in the field, oil pollution will have a long duration as shown by the untreated rocks and concrete blocks in this study (Fig. 5).

It is worth noting that TerraZyme™ has an excellent potential to biodegrade all of the ingredients of heavy oil (Hozumi *et al.*, 2000). The increased rate of removal of black-coloured oil from the rocks and concrete blocks treated with TerraZyme™ in this study predicts that if the oil coating on the substrates is fully treated within several months, a majority of the oil coating will be removed (Fig. 5). The biodegrading rate of the *Nakhodka* heavy oil is almost equivalent to the results of laboratory tests with TerraZyme™ (Hozumi *et al.*, 2000).

In previous studies on the application of the bioremediation agents to oil pollution, adding seed-cultures of petroleum-degrading bacteria have proven less promising for the promotion of biodegradation of oil than adding fertilizers such as Inipol EAP22™ and ensuring adequate aeration (Atlas, 1995), partly due to the technical difficulties in evaluating their impacts. However, the results of our collaborative research projects clearly show the effectiveness of the bioremediation

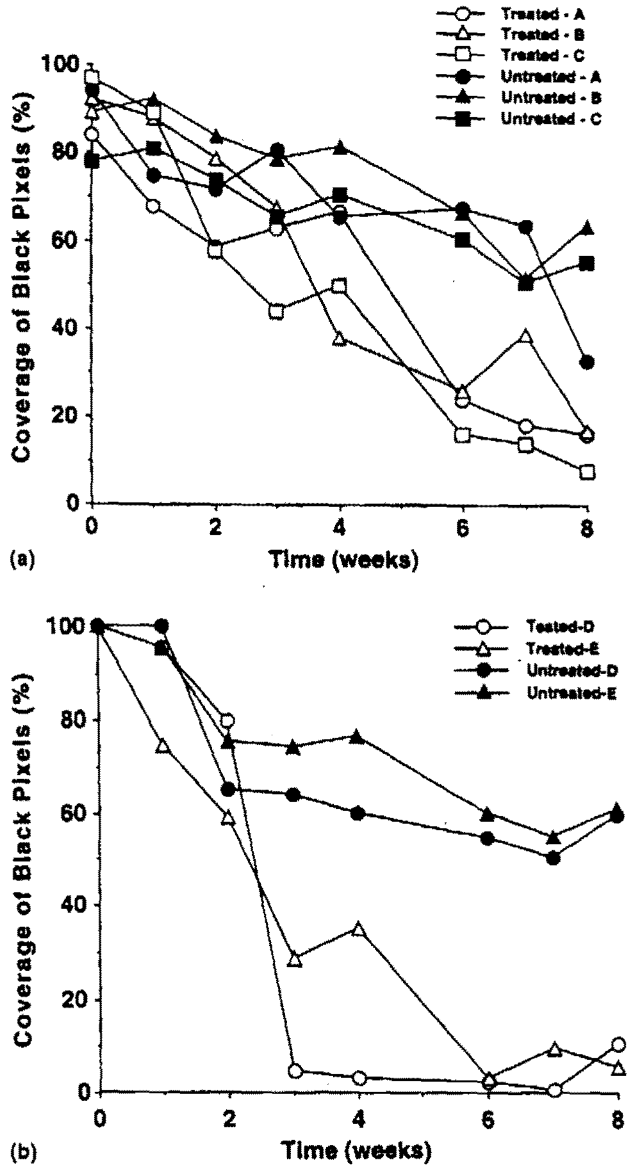

Fig. 5 The percentage of coverage of black pixels of Group 1 on the images of (a) rocks and (b) concrete blocks at the treated and untreated sites.

agent with microbiological cultures, TerraZyme™, on the enhancement of biodegradation of oil in both of in vitro (Hozumi *et al.*, 2000) and the field (the present study). In the latest 'National Contingency Plan Product Schedule (NCP)' published by the US Environmental Protection Agency (EPA) in February 1999, nine microbiological cultures for bioremediation of oil including TerraZyme™ (as the Oppenheimer Formula™) are noted in the products list for the treatment of oil spills (US EPA, 1999). They are multiple seed-cultures of natural petroleum-degrading bacteria.

At present, we need to revise their effectiveness as the bioremediation agents and to clarify the characteristics of their activities on the biodegradation of petroleum. We also need to study the combined use of both the fertilizers and seed cultures in treating oil in the field, since petroleum-degrading bacteria require specific additional nutrients for their growth (Lee and Levy, 1989, 1991). Actually, a seed-culture product, Alpha BioSea™ (Alpha Environmental), which was used to treat the oil spills of the *Mega Borg* in the Gulf of Mexico in 1990 (New Scientist, 1990; Mauro and Wynne III, 1990), and the *Apex* barge at Marrow Marsh along the Texas shoreline (Nadeau *et al.*, 1991), is a bacterial mixture and inorganic phosphorus and nitrogen nutrients (Swannell *et al.*, 1996). We need further studies in order to develop more effective bioremediation agents for the degradation of petroleum and to find their most efficient use in future large-scale oil spills around the world.

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